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CLADOCHYTRIUM ALISMATIS.

CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY. XLIX.

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(WITH PLATES II–IV)

While collecting at Glacialis pond, Cambridge, Mass., in October 1900, the writer found *Cladochytrium Alismatis* Büsg. on *Alisma Plantago* L. Although the season was so far advanced for this plant that all of the leaves were dead and in many cases broken off, still enough infected ones were obtained to show that the fungus was not uncommon in that locality last year. The discovery of the fungus is especially interesting since this is the first time it has been found in America, and also because the study of it revealed the possession of a peculiar temporary sporangial stage that it was not known to possess.

This Cladochytrium occurs on the leaf blades, petioles, and peduncles, forming lead-colored, generally subcircular sori 1–2^{mm} in diameter. These are generally distinct from one another, and on the woody tissues are apt to have more of an elongated shape. In the leaf blades the so-called spores, or resting sporangia, occupy the cells between the two layers of epidermis, producing a slight pustule which in the green leaves, as shown by *exsiccati* specimens, is surrounded by a rather inconspicuous discolored area. While the sori are long covered by the epidermis, in the dead leaves this eventually breaks open and the sporangia become scattered, thus leaving small circular holes in the parenchyma and pits in the woody tissues.

Although a temporary mycelium is developed in this genus, the fungus in this case was so far matured when discovered that no sign of it could be made out. From one to three or four sporangia were found in the parenchymal cells of the leaf blade (*fig. 33*), while in the more elongated cells of the peduncle there

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were often six or more arranged uniserially (*fig. 34*). The sporangia are oval to spherical, with sides sometimes slightly flattened, and vary from 25–45 μ in longest diameter. The cell wall is reddish-brown and rather thick, the exospore being smooth and prominent, and the endospore thin and hyaline. Attached to the exospore are often the remains of the plant cell. The contents of the sporangium consist of a more or less granular band of protoplasm adhering to the cell wall and a large central oil drop. In sporangia kept indoors for some weeks there seems to be a space between the oil drop and the protoplasmic band, though when young this is entirely occupied by the protoplasm in its more fluid condition.

Fischer (7) states that the sporangia of this fungus have never been germinated. The writer was successful in germinating the sporangia abundantly in the many trials made. When first collected a few sporangia would germinate in each trial, after being two or three days in a drop of water; but gradually it took longer and fewer germinated, until after they had been six to eight weeks indoors they ceased to germinate altogether. It was found, however, that if the infected leaves were placed in a small wire cage packed in damp moss and kept in a cool place the sporangia gradually assumed a condition favorable for germination when they were placed in a drop of water. Sporangia thus kept, even at the end of several months, germinated quite abundantly. Material, however, that had been placed outdoors at the same time in stoppered bottles and exposed to the cold of winter, but not to moisture, did not germinate very well. Most of the germinations were made in a drop of water in Van Tieghem cells at the ordinary room temperature.

The germination is similar to that given by Büsgen (5) for *Cladochytrium Butomi*. By osmotic action the sporangium becomes swollen until the exospore is broken open. This rupture is regular, a lid being split off in a circumscissile manner from about a third of the sporangium, which begins to crack at one side (*fig. 34*). The endospore meantime protrudes and eventually carries the lid at its top (*fig. 38*), or rarely bends it back as if on a hinge.

The exospore breaks open in so regular a manner that it indicates some definite line of dehiscence, though no such appearance was made out on the mature sporangia before germination. In the meantime there is a change in the contents. So far as could be determined, the large central oil drop is gradually eaten away from its circumference inward until it disappears, or is really broken up into a large number of very small drops scattered through the protoplasm. This latter is now seen to be very faintly divided into areas, showing the formation of the zoospores, in which the small oil drops frequently appear to be arranged more or less peripherally (*fig. 38*). These small oil drops gradually unite in each area to form the final large oil drop of each zoospore (*fig. 39*), and it is these oil drops more than any separating lines that indicate the number and position of the mature zoospores. While this has been going on, the papilla of dehiscence has been forming, though the sporangium is not always placed so that it can be seen. The papilla is best made out when examined in optical section at one side of the sporangium (*fig. 39*). It is usually a somewhat protruding area developed in the wall of the exposed endospore, and begins its dehiscence by the swelling of the cell wall which gradually extends inward as a hyaline area, due to the gelatinization of the wall. In some cases shortly before dehiscence this area seemed to be somewhat pitted, as is mentioned by Büsgen, perhaps allowing a greater flow of water into the sporangium, forming the extended hyaline space in this vicinity. In one sporangium, carefully watched, there could be seen no further change at this point, the hyaline space now having greatly extended inward, until there was a sudden movement of the mass of zoospores, and one coming out into this space darted toward the somewhat distended area in the endospore, and, apparently forcing its way through, escaped, and was followed, one by one by the others, about a hundred, until all had gone. This place of escape usually shows a polygonal opening with a few cracks extending from the angles (*fig. 37*).

The zoospores are somewhat variable in size and vary from ellipsoidal to spherical in shape. In most cases, however, while active they have the ellipsoidal form. Each one is provided with a prominent oil globule, usually protruding somewhat at one side, and a posterior cilium, three or four times its length, situated at the end nearer the oil globule. A denser mass of protoplasm can be seen on the other side of the oil drop (*fig. 40*). The zoospores are active from the first, moving with a swift-gliding motion for a short distance and then coming to rest, but soon moving on again, so that it is rather difficult to follow one for any length of time. With a high power the cilium can be made out readily when the zoospore is at rest. The movement is always in the direction of the end away from the cilium. The zoospores sometimes apparently remain active for twenty-four hours, and possibly under favorable conditions even longer. Sooner or later, however, they gradually grow more sluggish in their movements, eventually becoming rounded and losing their cilium (*fig. 42*). At this period they often exhibit slight amoeboid movements. They finally come completely to rest and the oil drop gradually disappears. Unless favorably situated they go to pieces, the process in the last stages being assisted by bacteria. A peculiar feature observed at this time was the appearance of a vacuole in which could be seen one or two irregular somewhat elongated protoplasmic bodies that kept up a constant vibration which did not seem to be merely Brownian motion (*fig. 43*).

After it was found that the resting sporangia of this fungus would germinate, it was thought desirable to see if further information concerning its life history could be made out. Accordingly some seeds of *Alisma Plantago* L. were gathered and germinated. This germination took place very slowly, requiring a preliminary soaking of the seeds in water for three or four weeks. The young seedlings first send out a filiform cotyledon, and the succeeding leaves for some time are merely phyllodia adapted for aquatic existence. These have chlorophyllous epidermal cells, but are so thin that they can be watched easily even under high powers of the microscope. In some of the experiments the plants were allowed to take root in soil covered with water, and the leaves removed as desired for examination. It was found, however, that even a piece of a leaf would remain fresh and active in a drop of water in a Van Tieghem cell for several days, and thus could be watched directly. The best results were obtained with young seedlings whose roots were placed in one of the arms of a Ward cell partly filled with a nutrient solution, and the first grass-like leaf was held up by a little cotton support so that it adhered to the cover glass containing a drop of water and the sporangia.

This species of Cladochytrium is placed by some botanists under the genus Physoderma, because it was not known to have a temporary sporangium. Our experiments were somewhat surprising, since they showed not only that such a stage existed, but that it was of a quite peculiar type. When this was first seen its relation to Cladochytrium was not suspected, but when it became evident that the two were connected, a large number of culture experiments were made in order to remove all doubt. The result was that this temporary stage appeared in every case where the resting sporangia germinated in water containing the young Alisma, and failed to appear in every case where the sporangia did not germinate and in the checks, both where nothing was placed with the young Alisma and where merely a piece of the old Alisma leaf which contained no sporangia was added.

As the zoospores are usually quite active for some time, it was impossible to follow a single one from the time of its exit from the sporangium until it came to rest. It was not difficult, however, when they began to grow sluggish in their movements, to find them settling down on the Alisma leaf and to watch them in their subsequent development, so that there was no doubt as to their identity. They almost always fix themselves on the epidermis just above the wall dividing two cells (*figs. 1–4*). Many do not succeed in penetrating the leaf, as the majority of those that were continuously observed during this stage failed to effect an entrance. In such cases the zoospores are sooner or later destroyed. In other cases, however, and usually within twenty-four hours after settling down permanently, a short rhizoid (*fig. 3b*) can be seen penetrating the cell. In the meantime the cilium has disappeared, and the oil drop has been growing smaller, being used apparently for the development of the rhizoid. This becomes somewhat club-shaped and soon develops from the swollen tip short branches which eventually grow out into slender branched and somewhat irregular threads (*figs. 16–18*). By means of this rhizoid the young sporangium, as it may now be called, gains nourishment and soon begins to increase in size, so that by the end of the first day after its penetration it may have grown to twice its former diameter. The chlorophyll grains of the host, which normally are distributed rather evenly over the cell wall, are now seen to be congregating in the vicinity of the rhizoid, so that very often all of them are eventually clustered there (*fig. 4*). Sooner or later the young sporangium ceases to swell uniformly and becomes constricted at places, so that it develops a number of folds. These give the elongated forms a somewhat bilateral, and the circular forms more of a radial symmetry. The sporangia keep on enlarging until sometimes they reach over 80 μ in diameter, though often they may increase but little beyond the size of the original zoospores. This variation is determined by condition of leaf, number of competing sporangia, etc. Usually the rhizoid is not extensively developed in the cell, so that after the sporangium begins to grow it is hidden from view.

After the temporary sporangium has attained its full size, its protoplasm becomes faintly divided into areas, about the circumference of which the numerous small oil drops seem to have chiefly congregated (*fig. 6*). In time the small oil drops of each area unite into the large drops of the individual zoospores, and it is by these, rather than by the appearance of visible boundaries, that the zoospores may be distinguished. While the zoospores have been forming, the papilla of dehiscence has been developed at one end or side of the sporangium. There is usually a space here free from zoospores, and the cell wall by gelatinization forms a distinct hyaline area extending inward in a manner similar to that already described in connection with the germination of the resting sporangia (*fig. 12*). At the time of dehiscence this gelatinized envelope is often protruded at the opening. Into this vesicle the zoospores dart, forming a ball, though it becomes dissolved and the zoospores scattered before all have escaped. The number of the zoospores that is produced depends on the size of the sporangium and varies from three or four up to probably as many hundred. The zoospores are like those formed in the resting sporangia except that they are possibly somewhat smaller. They settle down on the leaf in time, and give rise to secondary temporary sporangia, and even a third generation has been observed on the same leaf. Because of their crowded condition, the failing power of the leaf to furnish nourishment and its diseased condition, these second and third generations, at least in cell cultures, often produced sporangia of smaller size, and their zoospores seemed less active, in extreme cases even failing to move around after being discharged. Sometimes such zoospores had oil drops of a sickly reddish-yellow color. In leaves kept in cultures for some time, occasionally there occurred areas in which the sporangia covered half the surface of the leaf (*fig. 20*).

After a sporangium has discharged its zoospores, if the conditions for growth have been favorable, it is seen that a second sporangium has started within. The rhizoidal apparatus is separated from the sporangium by a septum, which, as soon as the zoospores have escaped, becomes protruded as a papilla, by the further growth of which the second sporangium is formed (*fig. 7*). This process may be repeated until in some few cases as many as five or six sporangia have been developed concentrically. Usually only one or two additional ones are formed, or even only the original one (*fig. 17*). They all dehisce at the same point, though this place of discharge often becomes obscured (*fig. 17*). The successive stages in the development of a temporary sporangium from a given zoospore derived from a resting sporangium are shown in *b*, *figs. 2–9*. The similar development of two small sporangia from the zoospores of a temporary sporangium is shown in *figs. 10–15.*

In a few cases, about three weeks after the first infection by the zoospores, there were also found mature resting sporangia similar to those found in the old leaves in the autumn (*fig. 46*). In one case the first sign of these was seen about two weeks after the first infection.

The development of the resting sporangia is quite similar to that described by Büsgen for *Cladochytrium Butomi.* The zoospores penetrate into the leaf much as they do for the formation of temporary sporangia, but in this case all of the contents of the zoospore passes into the enlarged end of the rhizoid-like process. This enlarges into a greatly swollen cell that eventually forms the *Sammelzellen* of authors (*fig. 25*). After this cell has reached its full size, a cell is separated by a partition across the smaller basal end (the end connected with the penetrating zoospore), which is less rich in oil globules than the larger apical or free end. This latter cell may also become divided into two or more, apparently sometimes temporary compartments. From this apical cell or cells are developed one to several very fine simple mycelial threads which connect the *Sammelzellen* to the cell walls of its host (*fig. 29*). On one side of the basal cell near the septum a rhizoid-like process is usually seen. In the same vicinity, evidently connected with this basal cell (though this was not surely made out) and more or less embraced by the rhizoid-like process, there appears a small round cell that rapidly enlarges into the sporangium, while the basal cell becomes empty of its contents (*figs. 27–29*). One or more of the mycelial threads may penetrate into another cell, and there give rise to a similar apparatus (*figs. 28–31*). In this way the fungus spreads from cell to cell. About this time the apical portion of the *Sammelzellen* becomes more or less collapsed (*fig. 29*), but whether this is due to the production of the secondary *Sammelzellen* developed from it, or to a process of fertilization, as claimed by some botanists, was not determined. On the side of the young sporangium there is also often seen a rhizoid-like process. The exact nature of these processes is not clearly shown, though very often they seem to bind the sporangial cell to the *Sammelzellen.* The sporangium while young contains numerous small oil globules scattered through the protoplasm, but these gradually unite into the one central oil drop. The sporangial walls also gradually thicken, become tinted, and the sporangium is finally matured (*fig. 46*). In the young sporangia there is some indication of a special place for the dehiscence of the cap. Young sporangia that were slightly crushed under a cover glass split off a cap on the side away from the *Sammelzellen.* This is evidently the side that in the mature sporangia is occasionally seen somewhat cupped. Mature sporangia, however, when crushed, do not always split open so regularly.

The infection experiments were all made with the aquatic leaves of seedling plants. It seems very likely that the nature of the leaves determines the character of the sporangia formed, the exposed temporary sporangia being adapted to the peculiar aquatic leaves, and the internal resting sporangia to the aerial leaves. There were some indications that the temporary sporangia did not develop so abundantly on the later leaves, while in none of the aquatic leaves did the mature sporangia develop to such an extent as one might expect if these presented the most favorable conditions for their production. What different conditions determined, as was sometimes the case, the production of both stages on the same leaf was not discovered, but a possible explanation may be that the resting sporangia were developed from zoospores that after settling on the leaf were exposed to the air.

The time relations in the development of the different stages of this fungus are shown approximately by the following observations made in one case:

March 21. Resting sporangia placed in water with young Alisma leaf.

March 23. Sporangia beginning to split off caps.

March 23–24. Active and quiet zoospores present.

March 25. Some zoospores have penetrated leaf to form young temporary sporangia.

March 26. Temporary sporangia of larger size.

March 28. Many temporary sporangia emptied of zoospores.

March 30. Oldest temporary sporangia emptied a second time; second crop of temporary sporangia developing.

April 1. Second crop of sporangia matured; third crop developing; signs of resting sporangia.

April 8. Several mature resting sporangia found.

Despite the luxuriant production of zoospores there are a number of causes that may prevent their forming sporangia. In the first place, they seem to be in their fresh condition a coveted prey for a number of the lower animals. In cell cultures amoebae were seen that had devoured eight or more. Even some of the little heliozoa, scarcely larger than a zoospore, were seen to catch as many as six before they became apparently satisfied. But the most ravenous enemies were occasional rotifers, which did not seem satisfied with a capture of several hundred. In the second place, the zoospores must reach their host and settle down upon it. Very likely many fail to do this. Finally, after reaching the host there are those that never succeed in penetrating into the tissues.

The resemblance of the temporary sporangia to the forms described by Zopf as *Rhizophyton*, on desmids and unicellular algae, suggested the possibility of the temporary sporangia developing on such hosts. In no case, however, where germinating resting sporangia were placed with various species of algae and desmids, were there any indications that the zoospores tried to penetrate them.

The discovery of the temporary sporangia for this Cladochytrium suggests the possibility that other members of the Physoderma section of the genus may also have a similar stage. Through the kindness of Professor Thaxter the writer succeeded in obtaining leaves of *Menyanthes trifoliata* infected with *Cladochytrium Menyanthis.* These leaves had been out doors all winter, and were badly rotted when obtained. The resting sporangia, however, germinated rather readily when placed in water. This Cladochytrium differs from the one on Alisma in the smaller, lighter-colored resting sporangia, which are also more permanently bound together by the cells of the host. Apparently it has also a very thin outer wall. Its germination differs decidedly in that no cap is split off. Instead, an elongation takes place at one end, as if the inner wall had pushed through the outer wall and the encumbering plant tissue. At the end of this prominent outgrowth is produced the papilla of dehiscence, and the subsequent history is similar to that of the other species (*figs. 21–24*). Attempts to infect young Alisma leaves and various algae with the zoospores from this species were not successful. Unfortunately, seeds or plants of the Menyanthes were not at hand for infection experiments.

Cladochytrium Alismatis was first described in 1833 in Germany by Wallroth (**1**), who named it *Physoderma maculare.* Its identity has since been fully established, as the original specimens of Wallroth were examined and figured by De Bary (**2**) in 1864. Fuckel (**3**) in 1869 transferred the fungus to the genus Protomyces, to which it evidently does not belong. In 1887 Büsgen (**5**) placed it in the genus Cladochytrium, and also changed the specific name to *Alismatis.* There was no apparent reason for this latter change, unless it was to make a uniform usage in his paper of having all the species named after their hosts. Strict adherence to priority would require the use of Wallroth's rather than of Büsgen's specific name. Schroeter (**4, 8**) and Berlese and De Toni placed the fungus under Physoderma, Schroeter distinguishing this from Cladochytrium by the absence of temporary sporangia. Fischer (**7**), on the other hand, does not consider them distinct, and places them with Schroeter's genus Urophlyctis under Cladochytrium.

The fungus has been found only on *Alisma Plantago* L. and its varieties. It is reported in Saccardo's *Sylloge Fungorum* as occurring in Germany, Finland, France, Italy, and Siberia. The following *exsiccati*, which have been examined by the writer, contain specimens: KRIEGER, Fungi saxonici, 681, *Physoderma maculare;* RABENH.-WINT.-PAZ., Fungi europaei, 3977, *Physoderma maculare;* SYDOW, Phycomyceten et Protomyceten, 45, *Cladochytrium Alismatis;* SYDOW, Mycotheca Marchica, 4331, *Cladochytrium Alismatis;* Sydow, Phycomyceten et Protomyceten, 141, *Physoderma maculare.* Numbers 4867 of Roumeguère's Fungi selecti exsiccati, 1417 of De Thümen's Mycotheca universalis, and 1 of Karsten's Fungi Fenniæ exsiccati, though thus named, are specimens of *Doassansia Alismatis.*

The writer wishes to acknowledge his indebtedness to Professor Thaxter for very helpful assistance in the study of this fungus, and to Professor Farlow for some of the references to the literature and the opportunity to examine herbarium material.

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EXPLANATION OF PLATES II–IV.

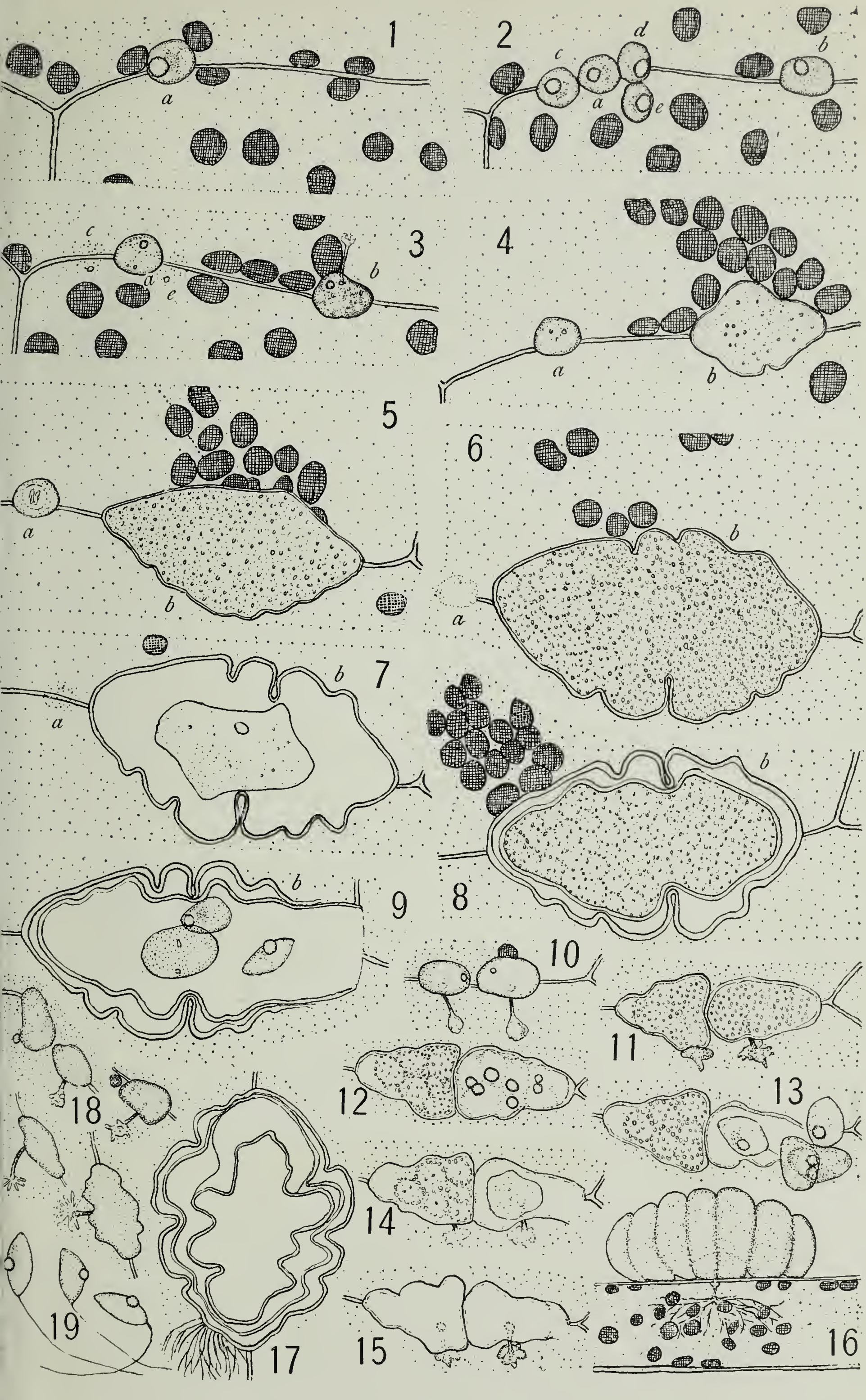
All figures magnified about 850 diameters, excepting *16, 17, 33–39* (about 550 diameters), and *20* (about 300 diameters).

PLATE II.

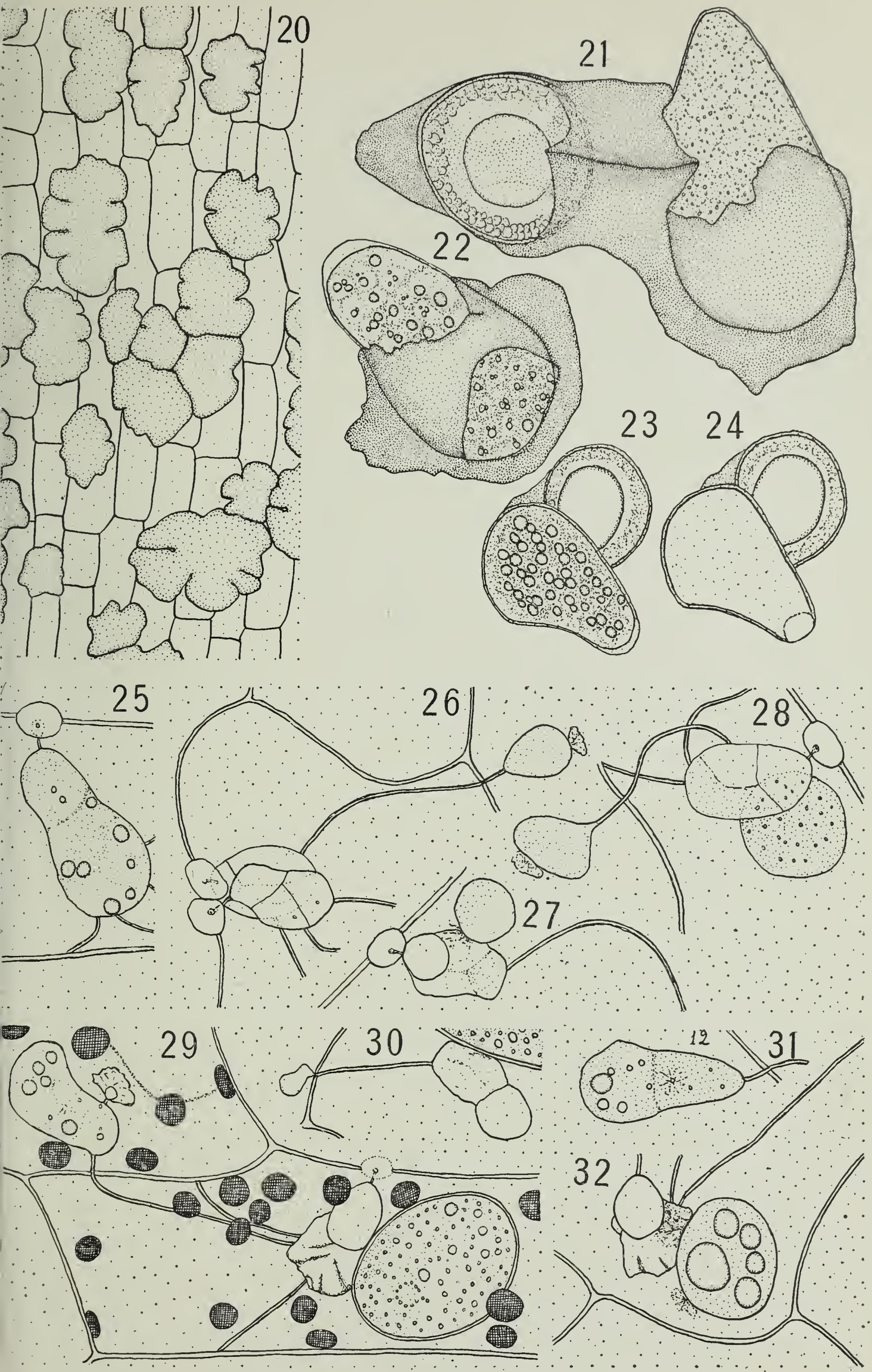
Figs. 1–20. Temporary sporangia of *Cladochytrium Alismatis.*

Figs. 1–9. Stages in the development of a temporary sporangium, produced from a zoospore of a resting sporangium, during nine consecutive days.

Figs. 10–15. Stages in the development of two small temporary sporangia, produced from zoospores of a temporary sporangium, during three successive days.



CLINTON on CLADOCHYTRIUM



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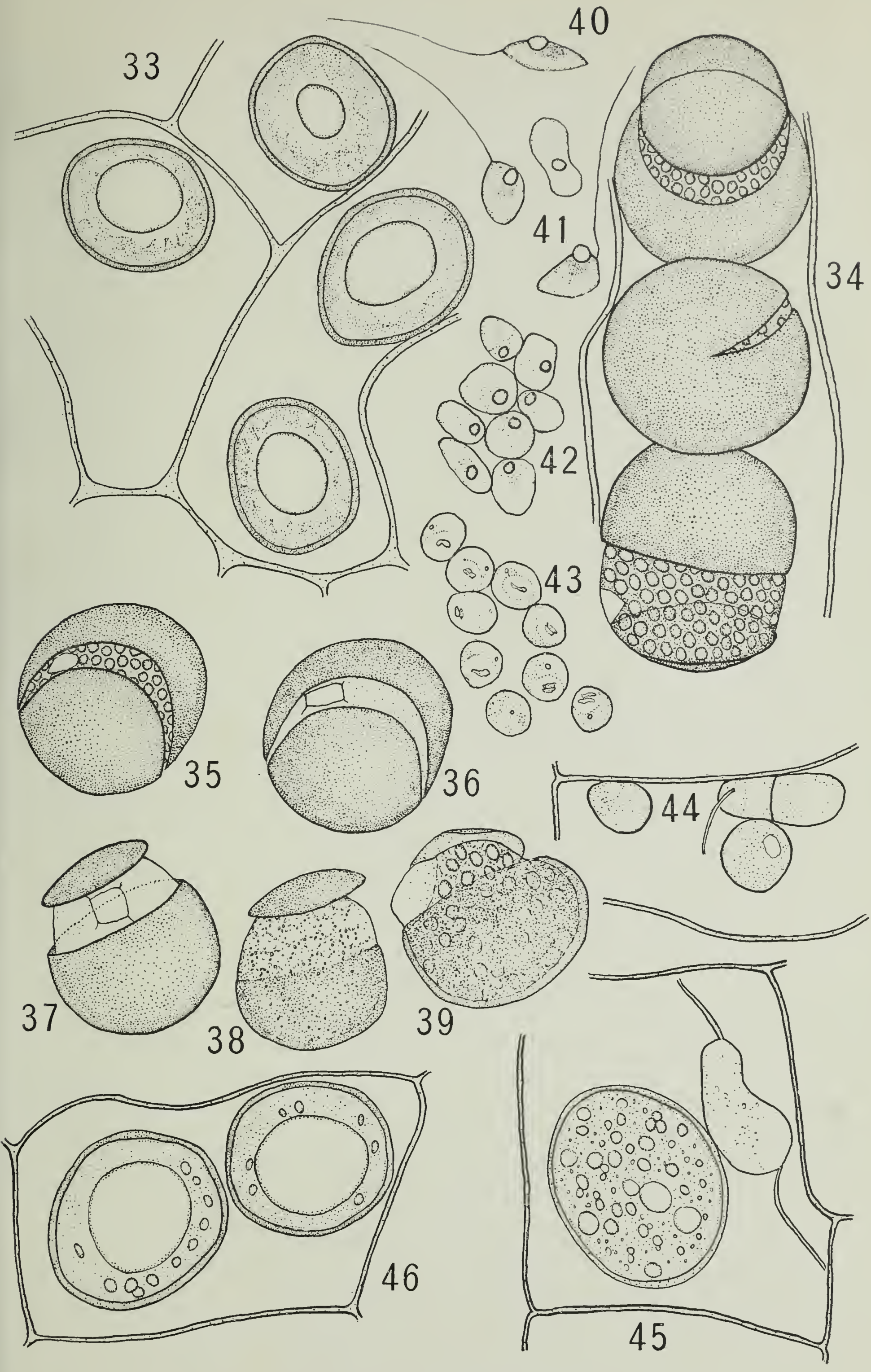

CLINTON on CLADOCHYTRIUM

FIG. 16. Side view of a temporary sporangium showing its rhizoid.

FIG. 17. A temporary sporangium that had emptied three times.

FIG. 18. Several young temporary sporangia, illustrating the early development of the rhizoid.

FIG. 19. Zoospores from temporary sporangium.

PLATE III.

FIG. 20. Epidermal view of portion of Alisma leaf showing numerous immature temporary sporangia.

FIGS. 21–24. Successive stages in the germination of *C. Menyanthis*, the sporangia being bound together by remains of plant cells.

FIGS. 25–32. Stages in the development of the resting sporangia of *C. Alismatis*.

FIG. 25. Penetrating zoospore with *Sammelzellen* and mycelial threads.

FIG. 26. Two penetrating zoospores with *Sammelzellen* and mycelial threads, one of which is forming a young *Sammelzellen* in an adjacent cell.

FIG. 27. *Sammelzellen* with attached young sporangial cell.

FIG. 28. Sporangial cell further advanced.

FIG. 29. *Sammelzellen* collapsing, sporangium about full size, and secondary *Sammelzellen* well advanced.

FIG. 30. The beginning of secondary *Sammelzellen*.

FIG. 31. Secondary *Sammelzellen* dividing into apical and basal cells.

FIG. 32. Secondary *Sammelzellen* collapsing, with attached young sporangium.

PLATE IV.

FIGS. 33–46. Resting sporangia of *C. Alismatis*.

FIG. 33. Section through cells of old leaf of Alisma, showing resting sporangia

FIG. 34. Germinating sporangia still imbedded in woody cell of petiole.

FIGS. 35, 36. The same resting sporangium just before and after emptying its zoospores.

FIG. 37. Side view of an empty resting sporangium.

FIG. 38. Resting sporangium with protoplasmic contents divided into areas, small oil drops, and beginning of papilla of dehiscence.

FIG. 39. Resting sporangium with prominent lateral papilla of dehiscence, about to discharge its zoospores.

FIG. 40. Fresh zoospore from resting sporangium.

FIG. 41. Older and sluggish zoospores.

FIG. 42. Still older zoospores, having lost their cilia.

FIG. 43. Old zoospores going to pieces, showing vacuoles with peculiar vibrating bodies.

FIGS. 44–46. Stages of resting sporangia in cells of young aquatic leaves of Alisma; produced by artificial infection.

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